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High dose interval vitamin D supplementation in pediatric patients with inflammatory bowel disease receiving Remicade

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Thesis

HIGH DOSE INTERVAL VITAMIN D SUPPLEMENTATION IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE RECEIVING REMICADE

by

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DEDICATION

I would like to dedicate this work to my dogs Chloe and Casey for bringing so much life and joy to my family in their years with us. We miss you both very much.

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I would like to thank Dr. Paul Rufo for giving me the opportunity to work with him this past year and for making every moment a teaching moment.

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ABSTRACT

BACKGROUND: Patients suffering from inflammatory bowel disease (IBD) are at increased risk of vitamin D deficiency. Daily or weekly vitamin D supplementation has not proven to be effective in improving vitamin D status, and it is thought that this failure has been primarily due to a lack of compliance. Circulating vitamin D is crucial to bone growth and development in children and adolescents. However, more recent data has demonstrated that vitamin D also plays a significant role in the maintenance and regulation of the immune system.

OBJECTIVES: The primary aim of this study is to investigate the safety and efficacy of administering high dose oral vitamin D therapy in pediatric patients with IBD. We chose to study patients receiving Remicade, an immunosuppressive monoclonal antibody therapy administered intravenously, as the need for scheduled hospital-based infusions provides a unique opportunity to ensure compliance in our study population.

METHODS: We identified consecutive pediatric patients with IBD with a recent 25-hydroxyvitamin D (25OHD) level < 30ng/mL, maintained on Remicade, and with no history of kidney or liver disease for inclusion in the study from November 2017 and

November 2018. Enrolled patients received one-year of open-label therapy. Vitamin D treatment doses were assigned by Remicade interval and patients received either 50,000 international units (IU) (every 4-5 weeks) or 100,000 IU (every 6-8 weeks) vitamin D_3 orally at the time of their Remicade infusions. In addition to vitamin D levels, spot urine calcium to creatinine ratios, serum calcium, phosphorus, and blood urea nitrogen (BUN) levels, quality of life metrics, and surveys pertaining to dietary vitamin D intake and ultraviolet B (UVB) radiation exposure were collected throughout the study period.

RESULTS: Baseline vitamin D status in enrolled patients did not differ by gender, dosing group, diet, or diagnosis (Crohn disease or ulcerative colitis). Subjects reached steady-state serum 25OHD levels after three doses administered over a span of 4 to 8 months, our data demonstrated an increase in average 25OH vitamin D levels from 21.17 ng/mL to 28.19 ng/mL in the 50,000 IU and 23.00 ng/mL to 33.18 ng/mL in the 100,000 IU dose groups, respectively. The improvement in vitamin D status did not correlate with changes in quality of life or disease activity. The response to vitamin D therapy was independent of diet, sun exposure, race, gender, diagnosis, or season of enrollment. There were no adverse events, including changes in urine calcium to creatinine excretion or serum BUN and creatinine values. Several patients manifest a small decrease in serum phosphorus during the initial phase of the study. However, these changes were transient and no subjects exhibited clinical signs or symptoms of hypophosphatemia.

CONCLUSION: High dose, interval vitamin D supplementation achieved steady-state 25OHD levels of 30 ng/mL or greater, with no signs of toxicity in patients enrolled in this pilot study. These data suggest that high-dose interval therapy may be a feasible

treatment option that bypasses limitations related to difficulties with patient compliance. Further studies are necessary to determine optimal dosage regimens and to assess endpoints related to immune function and improvements to gastrointestinal health.

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LIST OF ABBREVIATIONS

$1,25(OH)_2D$.....1, 25-dihydroxyvitamin D

25OHD.....25-hydroxyvitamin D

aPCDAI.....Abbreviated Pediatric Crohn Disease Activity Index

BCH .....Boston Children's Hospital

BMI..... Body Mass Index

BUN.....Blood Urea Nitrogen

Ca/Cr.....Urine calcium to creatinine ratio

CD..... Crohn Disease

CRP.....C-Reactive Protein

DBP.....Vitamin D-binding protein

ESR ..... Erythrocyte Sedimentation Rate

FGF23 .....Fibroblast growth factor 23

GI ..... Gastrointestinal

IBD.....Inflammatory Bowel Disease

IL-10 .....Interleukin-10

IU ..... International units

HIPAA .....Health Insurance Portability and Accountability Act of 1996

HRQoL……..... Health Related Quality of Life

NIDDK.....National Institute of Diabetes and Digestive and Kidney Diseases

PTH.....Parathyroid hormone

PUCAI.....Pediatric Ulcerative Colitis Activity Index

REDCap ..... Research Electronic Data Capture

TNFα ..... Tumor necrosis factor-α

UC ..... Ulcerative Colitis

UVB .....Ultraviolet B

VDR ..... vitamin D receptor

INTRODUCTION

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a collective term that refers to the chronic gastrointestinal (GI) inflammation and damage in patients with Crohn Disease (CD) and Ulcerative colitis (UC). CD can result in often discontinuous areas of inflammation that can occur in any section of the GI tract, extending from the mouth to the anus. In contrast, the inflammation observed in patients with UC typically begins in the rectum and extends, in contiguous fashion, to involve part or all of the large intestine. The inflammation in patients with CD is often transmural, and can extend to involve the whole wall of the intestine, while the inflammatory response in patients with UC is limited to the superficial lining of the large intestine. Patients with IBD typically present with a spectrum of GI-related and systemic symptoms including abdominal discomfort, fatigue, nausea, weight loss, fever, and anemia, all of which contribute to an overall decreased in quality of life (NIDDKa, n.d.; NIDDKb, n.d.). Similar to other chronic autoimmune or idiopathic inflammatory diseases like asthma, arthritis, and psoriasis, there is no cure for IBD. Instead, current treatment strategies are aimed at symptom relief, controlling inflammation, and preventing development of end-stage complications including fistulas, perforation, or impaired growth and development (NIDDKc, n.d.).

The prevalence of CD and UC among commercially insured pediatric patients in the U.S. has been estimated at 57.8 and 33.9 per 100,000, respectively. There appears to be an increased incidence of CD in the Northeast and Midwest regions, relative to the South and West regions (Table 1), and this has contributed to the hypothesis that average

exposure to sunlight may impact IBD prevalence or phenotype (Kappelman, Moore, Allen, & Cook, 2013a). Vitamin D deficiency has also been correlated with darker skin complexion, the winter months, and upper GI inflammation in CD (Pappa et al., 2006a). Data from related studies has supported what appears to be a strong association between IBD and vitamin D deficiency, with higher incidence of vitamin D deficiency (25OHD < 20ng/mL) or insufficiency (25OHD < 30 ng/mL) in patients with IBD than in control populations (Pappa et al., 2006a; Ahlawat, Weinstein, & Pettei, 2017). This has led to increased monitoring of vitamin D levels in IBD patients. Though these findings have raised awareness concerning vitamin D status in this disease population, most centers report only a 52.3% rate of compliance with at-home supplementation (Lewis et al., 2013).

Table 1. Prevalence of Pediatric IBD in the U.S. Incidence of CD and UC per 100,000 pediatric patients. Recorded by region in the U.S. from 2008 to 2009; *adapted from* (Kappelman, Moore, Allen, & Cook, 2013b).

	Age group	Region				
		Northeast	South	Midwest	West	Overall
	Pediatric (age <20 years)					
Crohn's Disease	Prevalence per 100,000 (95 % CI)[a]	65.1 (60.4–70.2)	54.1 (49.2–59.5)	61.4 (56.8–66.3)	43.2 (38.0–48.9)	57.8 (55.3–60.4)
Ulcerative Colitis	Prevalence per 100,000 (95% CI) [a]	38.3 (34.7–42.2)	33.4 (29.6–37.7)	31.5 (28.3–35.2)	31.1 (26.8–36.0)	33.9 (32.0–35.9)

CI Confidence interval

[a]Exact 95 % confidence limits were calculated using a variation on the Wilson Score confidence interval method

Vitamin D Deficiency

The role of vitamin D in calcium homeostasis and its relationship to bone diseases such as rickets and osteoporosis have long been established. However, the discovery of vitamin D receptor (VDR) expression in tissues outside of the bone/calcium axis has implicated vitamin D in the regulation of innate and adaptive immune responses (Yin & Agrawal, 2014a; Looman Kirsten I.M. et al., 2017). 25OHD is produced in the liver as a derivative of vitamin D_2 /D_3 obtained from dietary sources, in which they are absorbed in the small intestine and enter the circulation via the lymphatic system, and endogenously produced in the skin in response to exposure to UVB catalysis from sunlight. Once in circulation, the lipid soluble 25OHD is able to freely diffuse into tissue compartments, such as the kidneys, where vitamin D sensitive cells expressing 25OHD 1α-hydroxylase (CYP27B1) convert the vitamin to its active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which subsequently interacts with nuclear VDR's (Yin & Agrawal, 2014a). The binding of $1,25(OH)_2D$ to VDR in dendritic cells and T lymphocytes has been shown to have a net immunosuppressive effect, acting to suppress dendritic cell activation of lymphocytes, both directly, and by releasing cytokines, such as interleukin-10 (IL-10), that promote immunosuppressive T regulatory cell differentiation, and directly inhibiting T cell release of proinflammatory signals (Figure 1).

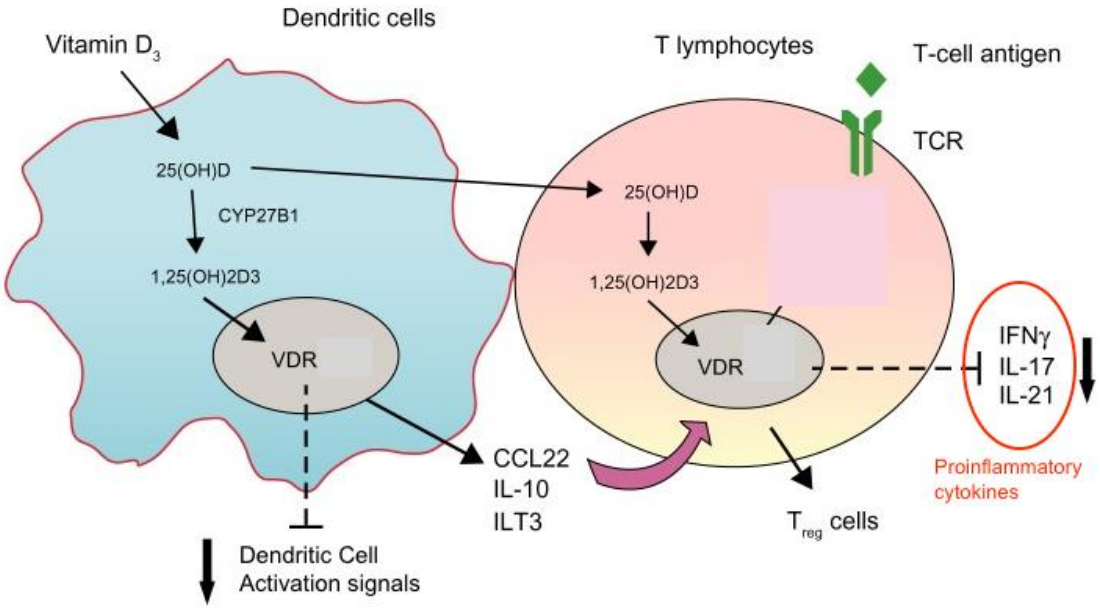

Figure 1. Immunosuppressive Action of Vitamin D on the Adaptive Immune Response. 1,25(OH)$_2$D binding to nuclear VDR following CYP27B1 activation of 25OHD works to 1) deactivate dendritic cells, 2) promote T regulatory cell (T_{reg} cells) differentiation via IL-10, and 3) inhibits T cell production and release of proinflammatory cytokines; *adapted from* (Yin & Agrawal, 2014a).

There is strong evidence from murine models that the actions of 1,25(OH)$_2$D and its cognate receptor (VDR) in the gut mucosa can play a significant role in mediating colonic inflammation. The expression of human VDR in murine intestinal epithelial cells was found to be protective against chemically induced and T cell transfer induced models of colitis, as well as able to rescue VDR knockout mice from colonic injury and inflammation (Liu et al., 2013). Mechanistically, it has been demonstrated that the lack of functional VDR signaling in VDR/IL-10 knockout mice leads to defective autophagy in the intestinal epithelium, altered Paneth cell function, and shifts in the microbiome composition toward dysbiosis and the development of colitis (S. Wu et al., 2015). While animal models of colitis point to a protective role of circulating 25OHD, support for an

analogous role in human disease is still lacking. Nonetheless, epidemiological studies have identified an inverse relationship between total 25OHD and intestinal inflammatory markers. Data from other studies has suggested that free/bioavailable vitamin D (which only represents about 5-10% of total circulating 25OHD levels), is relevant in regulating inflammation in newly diagnosed pediatric patients with UC (Garg, Rosella, Lubel, & Gibson, 2013; Sauer et al., 2018). Another study found vitamin D status to be predictive of duration of response to anti-tumor necrosis factor α (TNFα) therapy in patients with IBD, citing that patients with insufficient vitamin D levels (< 30 ng/mL) experienced earlier therapy cessation than patients who were vitamin D sufficient (Zator et al., 2014). In the general population, 25OHD levels greater than 40 ng/mL have been associated with a decreased risk of colon and colorectal cancers (K. Wu et al., 2007; Jenab et al., 2010), for which children with IBD are already susceptible (Olén et al., 2017).

Conclusions regarding the clinical significance of vitamin D beyond bone homeostasis is further complicated by differing definitions of vitamin D insufficiency and deficiency. A 2014 global, evidence-based consensus on the clinical management of nutritional rickets in children considers a serum 25OHD > 20ng/mL to be sufficient for turning off parathyroid hormone and protecting from adaptive hyperparathyroidism in the development of rickets (Munns et al., 2016). However, it has been suggested that serum 25OHD levels of 30-40 ng/mL are desirable for decreasing the risk of colorectal cancer in adult populations (McCullough et al., 2018).

Vitamin D Supplementation

Vitamin D is involved in multiple body systems, is derived from more than one source, and has a highly regulated metabolic pathway. As such, it is difficult to accurately assess the impact of any single intervention on circulating 25OHD levels. For example, higher rates of vitamin D deficiency and insufficiency have also been associated with obesity (Pereira-Santos et al., 2015). This is likely due to the high lipid solubility of vitamin D and obesity resulting in a much greater volume of distribution. Serum 25OHD levels can also vary based on the time of the year measurements were taken. One cross-regional, multi-year analysis of serum 25OHD measurements found that, on average, vitamin D levels display peak levels early in the Fall, about four weeks after maximal sun exposure, and are at their lowest in the early Spring, about four weeks after the winter solstice (Kroll et al., 2015a). Despite this annual cycle, recommendations from the FDA and the Institute of Medicine include a year-round dietary intake of 600 IU of vitamin D/day for children over the age of four, with vitamin D supplementation not to exceed 4000 IU/day (Institute of Medicine, 2011).

Although there are two forms of vitamin D, D_2 and D_3, vitamin D_3 has a greater affinity for the vitamin's carrier, vitamin D-binding protein (DBP), and has demonstrated a greater ability to influence the clinical indicator of vitamin D status, serum 25OHD (Armas, Hollis, & Heaney, 2004; Logan et al., 2013; Yin & Agrawal, 2014a). A one-time 100,000 IU dose of vitamin D_3 was shown to achieve peak 25OHD levels at one week after administration before 25OHD levels slowly returned to pretreatment levels over the next 12 weeks (Ilahi, Armas, & Heaney, 2008). While this single dose pharmacokinetic

study was able to achieve an average peak serum 25OHD level > 40.0ng/mL, a 15 ng/mL increase from pretreatment 25OHD levels, in subjects receiving just one dose of vitamin D_3, it is notable that 7% of subjects never attained a serum 25OHD level above 32.0 ng/mL. This latter observation could be explained by genetic heterogeneity among the enzymes and regulatory proteins involved in vitamin D metabolism.

The presence of small nucleotide polymorphisms in the CYP2R1 gene that encodes for 25OHD 25-hydroxylase, the enzyme responsible for converting D_2 and D_3 into 25OHD in the liver, has been implicated in the differential response to vitamin D supplementation. Patients found to have a lower activity polymorphism in the CYP2R1 gene were less responsive to vitamin D_3 supplementation, measured by change in serum 25OHD, relative to those patients without the polymorphism (Lewis et al., 2013).

Remicade (Infliximab) Infusions

IBD is a chronic GI inflammatory disease for which there is currently no cure. Pharmaceutical treatments focus on maintaining remission with oral or intravenous immunosuppressive and anti-inflammatory therapies, either as monotherapy or in combination. Infliximab, brand named Remicade (BLA 103772), is a chimeric monoclonal antibody that acts in circulation as a tumor necrosis factor-α (TNFα) antagonist. The antagonism results in attenuated immune cell proliferation, induction of as well as increased apoptosis in TNFα-secreting regulatory lymphocytes. Remicade presently holds FDA indication for treatment of Crohn disease and ulcerative colitis in both adult and pediatric patients 6 years and older who have not responded to other

therapies (Janssen Biotech, Inc., 2017). Patients receiving Remicade typically come in for infusions every four to eight weeks (Hyams et al., 2007). In most centers, lab work including complete blood cell count and blood chemistries are obtained at the time of each infusion.

Objectives

The aims of this study are to investigate the safety and efficacy of interval high-dose oral vitamin D supplementation in the treatment of vitamin D deficiency in pediatric patients with IBD. A secondary aim of the study is to explore the relationship between changes in serum 25OHD levels and clinical and biochemical markers of disease. Based on findings, this study will provide preliminary data that can be used to design and power larger and more definitive studies to assess the utility of interval vitamin D supplementation in pediatric patients with IBD.

METHODS

Screening

Patients carrying a diagnosis of IBD (CD, UC, or indeterminate colitis), followed in the Division of Gastroenterology at Boston Children's Hospital (BCH), and treated with Remicade were identified using the hospital electronic medical record. A subsequent chart review included abstraction of each patient's vitamin D status. Patients without a recorded serum 25OHD level later than October 1, 2016 were excluded from the study. Patients with a serum 25OHD > 30ng/mL were considered vitamin D sufficient and not considered currently eligible for the study. However, the serum 25OHD levels of vitamin D sufficient patients were re-evaluated each time there was an additional 25OHD level measured up until November 31, 2018. Patients with a serum 25OHD level < 30 ng/mL were classified as being vitamin D insufficient, but were only eligible to enroll if the date of approach/consent was within 8 weeks of the patient's most recent 25OHD level.

Patients were deemed ineligible if there was any history of granulomatous disease or deficits in kidney/liver function that might confound the evaluation of safety measures and vitamin D metabolism. Patients were also excluded for dysphagia, age > 25 years-old, and at the discretion of their primary GI provider.

Recruitment

Patients deemed eligible were approached for consent at a clinically scheduled Remicade infusion or GI outpatient follow-up appointment. Study patients were consented and enrolled after the goals, as well as the risk and benefits of the study were

discussed with patients and/or their guardian(s) and they had every opportunity to ask relevant questions.

High Dose Vitamin D Supplementation

Upon enrollment, patients were assigned to the 50,000 IU dose group if their clinical plan called for Remicade infusions every 4 to 5 weeks or to the 100,000 IU dose group if their clinical plan called for Remicade infusions at intervals of six to eight weeks. Patients were administered either one capsule (50,000 IU dose group) or two capsules (100,000 IU) of vitamin D_3 at their next Remicade infusion, deemed "Infusion 1," and each subsequent Remicade infusion thereafter for about one year.

The D3-50 50,000 IU vitamin D_3 capsules (Bio-Tech Pharmacal, Inc., Fayetteville, AR) were used for supplementation. Other ingredients included microcrystalline cellulose and gelatin.

Survey and Lab Data

Patients were asked to fill out the IMPACT-III instrument, a 35-item health-related quality of life (HRQoL) assessment for pediatric patients with IBD (Griffiths et al., 1999; Ogden et al., 2008). At Infusion 1, the date of first high dose vitamin D administration, patients were asked to fill out a baseline survey (Appendix A) for use in estimating a particular patient's average sunlight/UVB exposure and dietary vitamin D intake. The electronic medical record was also used to abstract the most recent assessment of clinical disease activity, recorded as either Abbreviated Pediatric Crohn

Disease Activity Index (aPCDAI) or Pediatric Ulcerative Colitis Activity Index (PUCAI) scores (Turner et al., 2007; Kappelman et al., 2011), surgical history, relevant diagnostic studies, and concurrent medications. At each subsequent infusion (typically administered every 4 to 8 weeks), patients filled out a follow-up survey (Appendix B) to assess extra-supplemental sources of vitamin D. At each infusion, anthropomorphic measures, changes in concurrent medications, clinical care updates, and hematology and blood chemistry labs, that make up care for all patients receiving Remicade, were collected (Table 2).

A serum 25OHD and spot urine calcium to creatinine ratio (Ca/Cr) was determined at every other infusion to evaluate efficacy and to monitor for hypercalciuria (Jones et al., 2012), respectively. If a patient was found to have an elevated spot urine calcium to creatinine ratio (Ca/Cr >0.200), a follow-up spot Ca/Cr was ordered to be completed at the time of their next infusion (four to eight weeks later). If a patient was observed to have two subsequent spot Ca/Cr ratios >0.200, a 24-hour urine collection was ordered, Ca/Cr calculated, and the propriety of continued participation in the study was assessed.

Between 48 and 64 weeks after first high dose administration (Infusion 12 for the 50,000 IU dose group and Infusion 8 for the 100,000 IU dose group), patients were asked to again fill out the IMPACT-III healthy related quality of life survey and disease activity was evaluated using the aPCDAI or PUCAI scores (Table 2).

Table 2. Data collection schedule. Subjects were followed for 48-64 weeks, depending on duration between intervals. Subjects receiving 100,000 IU dose completed study at Infusion 8 while those receiving 50,000 IU were followed until Infusion 12. When appropriate, date of consent was concurrent with Infusion 1.

Item	**Date of Consent**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9** 50K only	**10** 50K only	**11** 50K only	**12** 50K only
Consent Form	X												
IMPACT-III (HRQoL)	X								X 100K only				X
Baseline Survey		X											
Clinical Care Labs		X	X	X	X	X	X	X	X	X	X	X	X
Follow Up Surveys			X	X	X	X	X	X	X	X	X	X	X
High Dose Vitamin D_3		X	X	X	X	X	X	X	X	X	X	X	X
Serum 25OHD			X		X		X		X		X		X
Spot Urine Ca/Cr ratio			X		X		X		X		X		X

Data Collection and Analysis

Abstracted laboratory and clinical data were transferred into Case Report Forms created on Research Electronic Data Capture (REDCap), a Health Insurance Portability and Accountability Act of 1996 (HIPAA)-compliant web application. Data exported from REDCap was then analyzed in R Studio, an open-source computing and graphics platform.

RESULTS

Enrollment

A total of 512 pediatric patients with IBD receiving Remicade were screened for serum 25OHD status between October 2017 and November 2018. 386 (75.4%) had serum 25OHD levels measured between October 1, 2016 and November 31, 2018. Of these, 188 (48.7%) had serum 25OHD levels > 30ng/mL and were considered vitamin D sufficient. Of the 198 (51.3%) patients with serum 25OHD levels < 30ng/mL, 46 (23.7%) were ineligible to approach due to the lack of a 25OHD level that would be within 8 weeks of the date of consent. Screening for pre-existing liver, kidney, and granulomatous disorders excluded 14 patients, leaving 134 patients eligible to approach. 77 (56.7%) of these patients would be available for the expected duration of the study, were deemed appropriate for the study by their primary GI providers, and were approached, 8 declined and 69 were consented. However, 3 patients were unable to swallow the study capsules and 1 was found to be allergic to gelatin, an inactive ingredient in the capsule formulation. These 4 patients were not enrolled. Of the 65 patients enrolled, 4 were excluded for noncompliance with Remicade infusions or inconsistently taking study capsules, 2 were withdrawn by their providers in order to return to prescribed supplementation, 1 opted to withdraw from the study as a result of nausea that they experienced in conjunction with vitamin D_3 dosing, 4 patients were either transferred to adult care or switched to a maintenance medication other than Remicade, and 1 patient lost to follow-up, resulting in final enrollment of 53 patients (Figure 2).

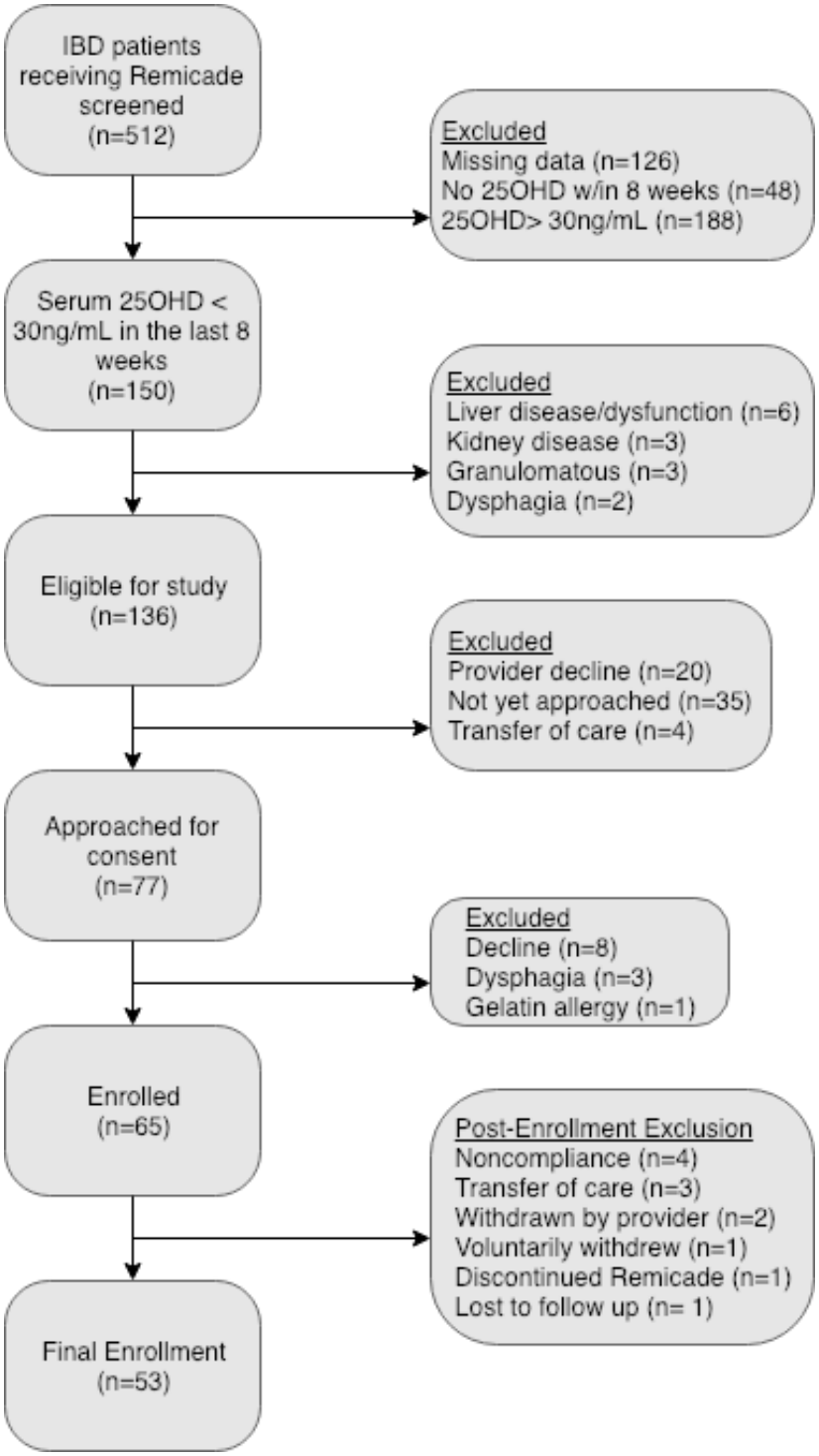

Figure 2. Subject Screening and Recruitment. A total of 512 patients receiving regular Remicade infusions for IBD at BCH were screened for vitamin D status and study eligibility between Oct. 2017 and Nov. 2018. A total of 65 patients were enrolled, 12 were excluded post-enrollment to give final enrollment of 53 subjects.

Demographics

65 patients were enrolled, but 5 patients were removed from the study before completing the baseline surveys or receiving a second dose and 7 were either retroactively removed or lost to follow up after receiving 3 or more doses. Therefore, baseline and dose response data were available from 60 subjects in both dosing groups.

The total enrolled subject population was 68% male, distributed similarly in the 50,000 IU (66.72% male) and 100,000 IU (70.7% male) dose groups, with an average age of 16.50 ± 3.83 years at time of consent. The 50,000 IU was slightly younger (15.00 ± 4.59 years) than the 100,000 IU (17.14 ± 3.32 years), and the total subject population had an average body mass index (BMI) of 23.9 ± 5.7 kg/m^2 at time of consent. The majority of study subjects identified as White (70%), 21.7% identified as Other, 6.7% as Black, 1 subject identified as Asian, and 1 as Native American. This distribution was similarly reflected in each dosing group. 45 (75%) of the enrolled subjects carried a diagnosis of CD, 14 (23%) of UC, and 1 subject was classified as having indeterminate colitis. All patients participating in the study received regular Remicade infusions as a maintenance therapy for IBD and for 26 (43.3%) this was their only maintenance therapy. 22 (36.7%) patients were prescribed an immunomodulator as adjunctive therapy (either methotrexate or mercaptopurine), 4 (6.7%) took an aminosalicylate adjunct, 3 (5%) were currently prescribed steroids at time of consent, and 5 (8.3%) were treated with a combination of two or more of these therapies. The 100,000 IU dose group had more subjects not on additional IBD therapies (48.8%) and less multiple adjunctive therapies (2.4%) in relation to the 50,000 IU dose group, 31.6% and 21.1%, respectively (Table 3).

Table 3. Demographics and Descriptive Characteristics. Description of the 60 patients enrolled in the study who completed baseline data and received 3 or more doses of high dose supplementation, separated by dose group.

	50,000 IU dose group	*100,000 IU dose group*	*Total*
Gender			
Male	12 (66.7%)	29 (70.7%)	41 (68%)
Female	6 (33.3%)	13 (31.7%)	19 (32%)
Age (years) *At time of consent*	15.00 ± 4.59	17.14 ± 3.32	16.50 ± 3.83
Ethnicity			
White	12 (63.1%)	30 (73.2%)	42 (70.0%)
Black	1 (5.3%)	3 (7.3%)	4 (6.7%)
Asian	0	1 (2.4%)	1 (1.7%)
Native American	1 (5.3%)	0	1 (1.7%)
Other	5 (26.3%)	8 (19.5%)	13 (21.7%)
Diagnosis			
CD	14 (73.7%)	33 (80.5%)	45 (75.0%)
Inflammatory	8	23	31
Penetrating	1	2	3
Stricturing	3	5	8
Unknown	0	3	3
UC	5 (26.3%)	9 (22.0%)	14 (23.3%)
Proctitis	0	0	0
Left-sided	1	2	3
Pancolitis	4	7	11
Indeterminate colitis	1 (5%)	0	1 (1.7%)
Concurrent IBD Medications			
Immunomodulator	7 (36.8%)	15 (36.6%)	22 (36.7%)
Aminosalicylates	1 (5.3%)	3 (7.3%)	4 (6.7%)
Steroids	0	3 (7.3%)	3 (5.0%)
≥2 of the above	4 (21.1%)	1 (2.4%)	5 (8.3%)
Remicade only	6 (31.6%)	20 (48.8%)	26 (43.3%)
BMI (kg/m^2) *At time of consent*	25.0 ± 5.0	23.5 ± 5.9	23.9 ± 5.7
Baseline serum 25OHD (ng/mL)	21.17 ± 5.70	23.00 ± 4.07	22.45±4.67

Vitamin D Status

All patients enrolled had been identified as vitamin insufficient or vitamin D deficient as part of recruitment screening and had recent total 25OHD levels of less than 30 ng/mL. The average pretreatment serum 25OHD level for male subjects (21.73 ± 4.91 ng/mL) was not shown to differ from that of female subjects (24.00 ± 3.73 ng/mL) by an unequal variance *t*-test (*t*, -1.97; confidence interval [CI], -4.58 – 0.05; $p = 0.06$). Unequal variances *t*-test also did not suggest a difference in pretreatment vitamin D status between patients with CD and UC, 22.22 ± 4.74 and 23.02 ± 4.68 respectively (*t*, -.056; CI, -3.78 – 2.17; $p = 0.58$). A one-way ANOVA of subjects' responses to survey questions showed differences in the baseline 25OHD level based on racial identification ($F[1, 55]= 6.77$; $p< 0.001$), with visualization of this data showing that subjects who identified as 'Other' had, on average, lower baseline 25OHD levels than those who identified as 'White,' 'Black or African American,' 'Asian,' 'Native Hawaiian or Pacific Islander,' or 'American Indian or Alaskan Native.' The severity of vitamin D deficiency was not shown to correlate with age (Pearson R [R], -1.24; CI, -0.36 – 0.13; $p = 0.34$), BMI (R, -0.09; CI, -0.34- 0.16; $p = 0.46$), or inclusion of dairy, vitamin D fortified cereals, and fish in the diet (R, -0.07; CI, -0.33- 0.21; $p = 0.64$) in this vitamin D deficient/insufficient population. In regards to skin exposure to sunlight and UVB radiation, baseline vitamin D levels appeared independent of reported sun sensitivity ($F[1, 55]= 0.60$; $p= 0.44$) and time spent outside on a typical day (R, -0.02; CI, -0.28- 0.25; $p= 0.90$). However, increased skin exposure to the sun, reported as daily typical clothing coverage and ranging from just head and neck to "bathing suit" exposure, was

positively correlated with total 25OHD levels among vitamin D deficient patients (R, 0.44; CI, 0.20 – 0.63; $p > 0.001$). Although seasonal variation in serum 25OHD levels has been demonstrated in populations living at higher latitudes (Kroll et al., 2015b), there was no observed difference in baseline 25OHD level based on the month of treatment initiation ($F[9, 50]= 1.345$; $p = 0.24$).

All 60 patients were treated, or are currently being treated, according to the supplementation protocol at their Remicade infusions for one year. Both dosing groups, on average, reached steady state serum 25OHD levels after administration of three doses, which was about four to eight months after the start of therapy, and therefore total 25OHD measured at infusion 4 was designated the steady-state level (Figure 3). Steady-state serum 25OHD levels were 28.91 ± 5.60 ng/mL and 33.18 ± 5.80 ng/mL for the 50,000 IU and 100,000 IU dosing groups, respectively. In general, total serum 25OHD levels were increased from baseline levels in both the 50,000 IU dosing group (*t*, 6.932; CI, 5.20 – 9.79; $p < 0.01$) and the 100,000 IU dosing group (*t*, 8.722; CI, 7.72 – 12.41; $p < 0.01$) by paired t-test analysis. The 25OHD increase from baseline was 7.74 ng/mL in 50,000 IU dose group and 10.18 ng/mL in 100,000 IU dose group, but these changes were not found to be statistically different (*t*, -1.626; CI, -5.76 – 0.61; $p = 0.11$). This change in vitamin D status was found to be similar between CD and UC patients (*t*, -1.398; CI, -8.29 – -1.79; $p= 0.19$) and between genders (*t*, 1.60; CI, -0.63- 5.50; $p = 0.12$).

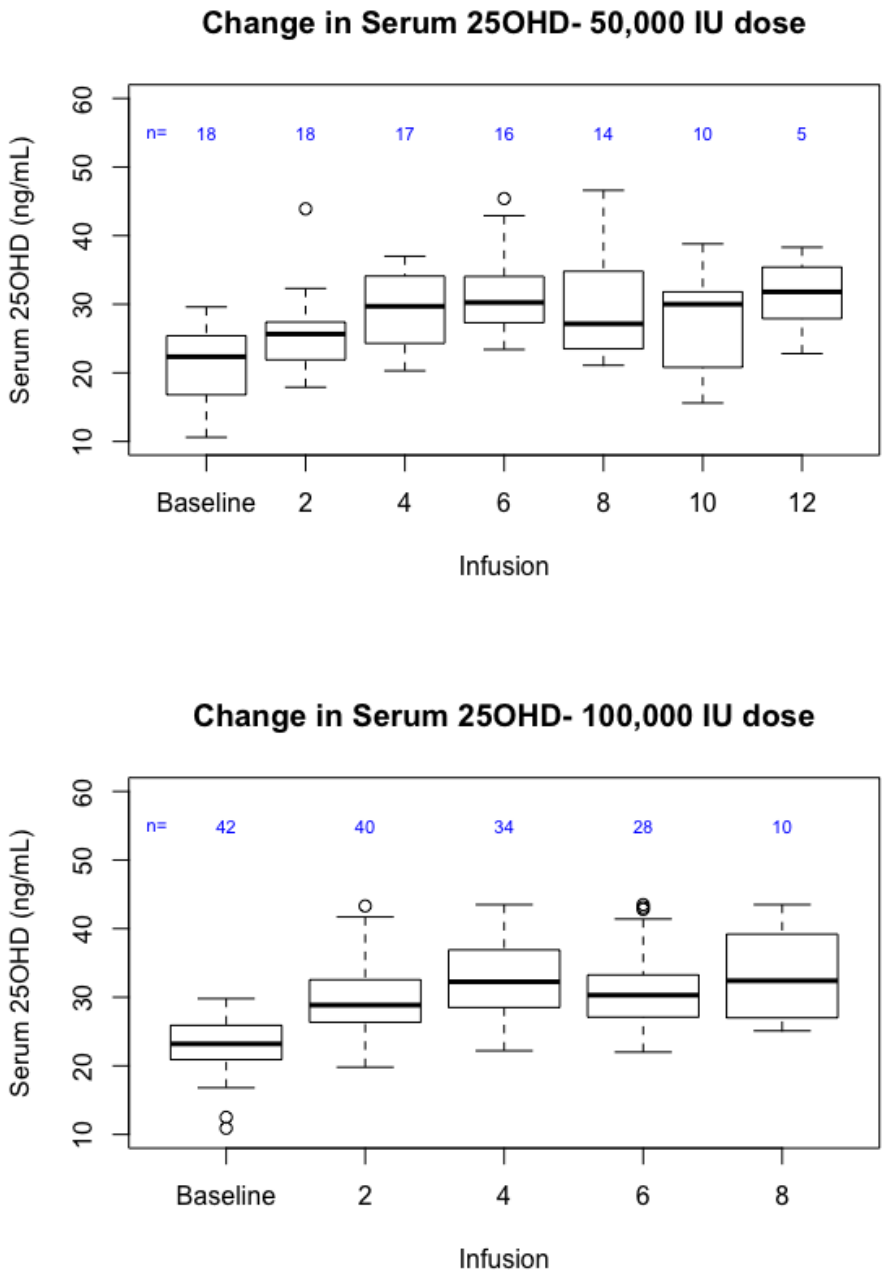

Figure 3. Effect of high dose therapy on serum total 25OHD. 60 patients were treated with high doses oral vitamin D_3 concurrent with Remicade infusions for approximately one year. A steady state 25OHD level was achieved after 3 doses was achieved in both the 50,000 IU dosing group *(top)* and the 100,000 IU dosing group (*bottom).*

Data from recent studies on vitamin D status in adults with IBD suggests that achieving total circulating 25OHD levels in the 30-40ng/mL range can be protective against colon cancer. By infusion 4 of the study, a total of 31 (61%) of subjects had achieved total 25OHD ≥ 30 ng/mL and were considered to be vitamin D sufficient. The percentage of subjects achieving target range 25OHD levels at steady state did not differ by dosing group: 8 subjects (47%) receiving 50,000 IU doses reached 25OHD ≥ 30ng/mL in 16 to 20 weeks, and 23 subjects (68%) receiving 100,000 IU doses achieved steady state 25OHD levels ≥ 30ng/mL in 24 to 48 weeks ($\chi^2[1, N=51] = 1.24$; $p = 0.26$). There was neither a difference in response with respect to the different types of IBD, as 21 (52.5%) subjects and 9 (90%) subjects achieved sufficient 25OHD at steady state with CD and UC, respectively ($\chi^2[1, N=50] = 3.25$; $p= 0.07$), nor a difference between genders ($\chi^2[1, N=51] = 0.06$; $p = 0.81$).

Throughout the course of the study, each subjects' clinical course, lab values, medication changes, diet, and seasonal sun exposure were observed in order to determine extra-supplemental factors that might also influence total 25OHD. The time of year was not found to affect steady state 25OHD levels achieved (F [8, 42] = 1.062; $p = 0.41$) or change in 25OHD from baseline to steady-state (F [8, 42] = 1.193; p =0.33) (Figure 4). In addition to tracking vitamin D status, blood chemistry and inflammatory markers measured as part of patients' standard clinical care were also monitored to assess safety and efficacy.

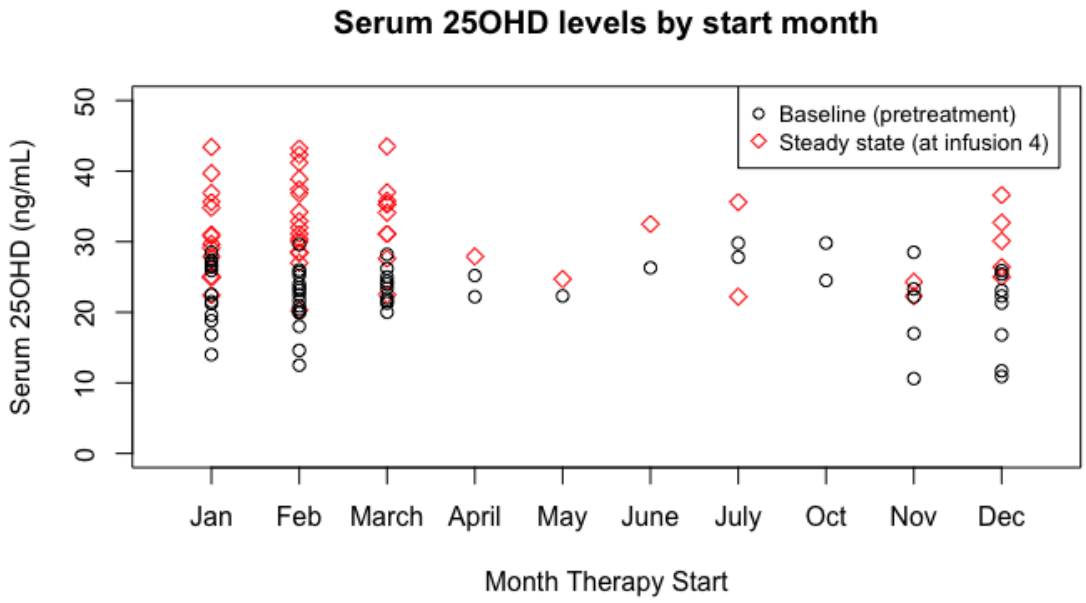

Figure 4. Effect of the season of therapy initiation on total 25OHD levels. Subjects were started on high dose therapy within 8 weeks of baseline 25OHD level throughout the year. The month of therapy initiation was not found to influence baseline levels, nor was it found to affect steady-state levels, defined as 25OHD level at Infusion 4, or change in 25OHD from baseline to steady-state.

In addition to monitoring seasonal effects, survey data were collected throughout the study to assess extra-supplementation sources of vitamin D, most notably diet and sun exposure. Total change in serum 25OHD between was not shown to correlate with age or BMI for either dosing group. There was also no demonstrated influence of race, self-reported sun sensitivity, hours spent outside, or areas of skin exposure on serum 25OHD in patients receiving high dose supplementation. Reported consumption of vitamin D rich foods, such as dairy, vitamin D fortified cereals, and fish was not shown to correlate with changes in total 25OHD from baseline in the 100,000 IU dosing group (Table 4). The effect of diets in the 50,000 IU group remains undetermined due to low sample size.

Table 4. Effect of External Factors Affecting Vitamin D Status and Therapy Response. Vitamin D status can be influenced by a multitude of factors including dietary (exogenous) intake, UVB catalyzed production (endogenous), body composition, and liver and kidney function. Data was collected from patients on dietary intake and UVB radiation exposure throughout the study to assess their influence on total 25OHD. **Low sample size, n= 3*

	50,000 IU					**100,000 IU**			
	Pearson R	*95% CI*	*F value*	*p-value*		*Pearson R*	*95% CI*	*F value*	*p-value*
Age (years)	0.18	-0.33 to 0.61		0.50		-0.12	-0.44 to 0.23		0.50
BMI (kg/m^2)	0.01	-0.50 to 0.49		0.96		-0.09	-0.42 to 0.26		0.61
Self-identified race			1.01	0.42				0.56	0.65
Relative dietary Vitamin D	0.93*			0.24		-0.21	-0.6 to 0.29		0.41
Self-reported sun sensitivity (Hi, Med, or Lo)			0.01	0.92				0.05	0.82
Time spent outside (hrs/day)	0.50	-0.02 to 0.80		0.06		0.25	-0.12 to 0.56		0.17
Skin area exposed to the sun (relative clothing coverage)	-0.22	-0.66 to 0.33		0.43		0.37	0.01 to 0.64		0.05

Safety

Blood chemistry labs were traced to assess for the emergence of side effects that could be related to interval high dose vitamin D treatment, particularly hypercalcemia and risk of kidney stones. Blood chemistry labs remained unchanged through four

administered doses in the 50,000 IU dosing group, and no change from baseline was detected in serum calcium (F[3, 39] = 0.23; p = 0.87), creatinine (F[3, 39] = 1.54; p = 0.22), phosphorus (F[3, 39] = 0.33; p = 0.81) or BUN (F[3, 39] = 0.60; p = 0.62) by one-way repeated measures analysis of variance. The 100,000 IU dosing group also saw no change in calcium (F[3, 87] = 0.35; p = 0.79), creatinine (F[3, 87] = 1.86; p = 0.14), or BUN (F[3, 87] = 0.18; p = 0.91) lab values. However, a fall in serum phosphorus levels (F[3, 78] = 3.52; p = 0.02) was observed by the fourth dose in 100,000 IU dose group (Table 5). Spot urine samples were also collected to assess the risk of hypercalciuria and the development of kidney stones while on high dose supplementation.

Table 5. Blood chemistry to assess therapy safety. Blood chemistry labs were observed to monitor occurrence of hypercalcemia or other vitamin D metabolism disturbances as a result of therapy. There was no remarkable effect of high dose therapy on CHEM10 levels.

mg/dL	*Baseline*	*Infusion 2*	*Infusion 4*	*Infusion 6*	*Infusion 8*	*Infusion10*	*Infusion12*
50K							
Calcium	9.26± 0.45	9.25± 0.19	9.19± 0.25	9.31± 0.20	9.35± 0.34	9.30±0.46	9.18±0.35
Creatinine	0.63± 0.24	0.62± 0.21	0.62± 0.22	0.63± 0.19	0.67± 0.21	0.66±0.18	0.51±0.09
Phosphorus	3.82± 0.64	3.95± 0.81	3.78± 0.87	3.85± 0.77	3.91± 0.90	3.99±0.68	4.33±0.49
BUN	11.5± 5.3	10.9± 3.7	11.2± 2.7	10.4± 3.7	9.7± 2.8	10.8±1.8	11.8±1.8
100K							
Calcium	9.34± 0.33	9.30± 0.33	9.33± 0.27	9.37± 0.34	9.48± 0.25	--	--
Creatinine	0.71± 0.17	0.71± 0.17	0.73± 0.19	0.74± 0.17	0.76± 0.22	--	--
Phosphorus	3.78±0.57*	3.81±0.50*	3.53±0.57*	3.69±0.51	3.94±0.57	--	--
BUN	11.6± 3.9	12.2± 4.4	11.5± 3.7	12.2± 3.8	11.0± 2.4	--	--

While spot urine calcium to creatinine ratio was not collected at baseline, urine Ca/Cr's were measured for the duration of therapy to monitor occurrences of hypercalciuria. The average urine Ca/Cr for both dosing groups did not exceed 0.200 once steady state 25OHD levels had been achieved, and beyond. One-way repeated

measures analysis of variances did not suggest any change in urine calcium to creatinine ratios in the 50,000 IU dosing group ($F[2, 30] = 0.02$; $p = 0.98$) or the 100,000 IU dosing group ($F[2, 38] = 2.32$; $p = 0.11$) up to and after reaching steady state 25OHD levels, from the second through the sixth infusions (Figure 5). However, it is noted that, of the subjects with spot Ca/Cr ratios up to infusion 6, there were 1 and 5 urine Ca/Cr ratios considered abnormal (>0.200) in the 50,000 IU dosing group and 100,000 IU dosing group, respectively (Table 6).

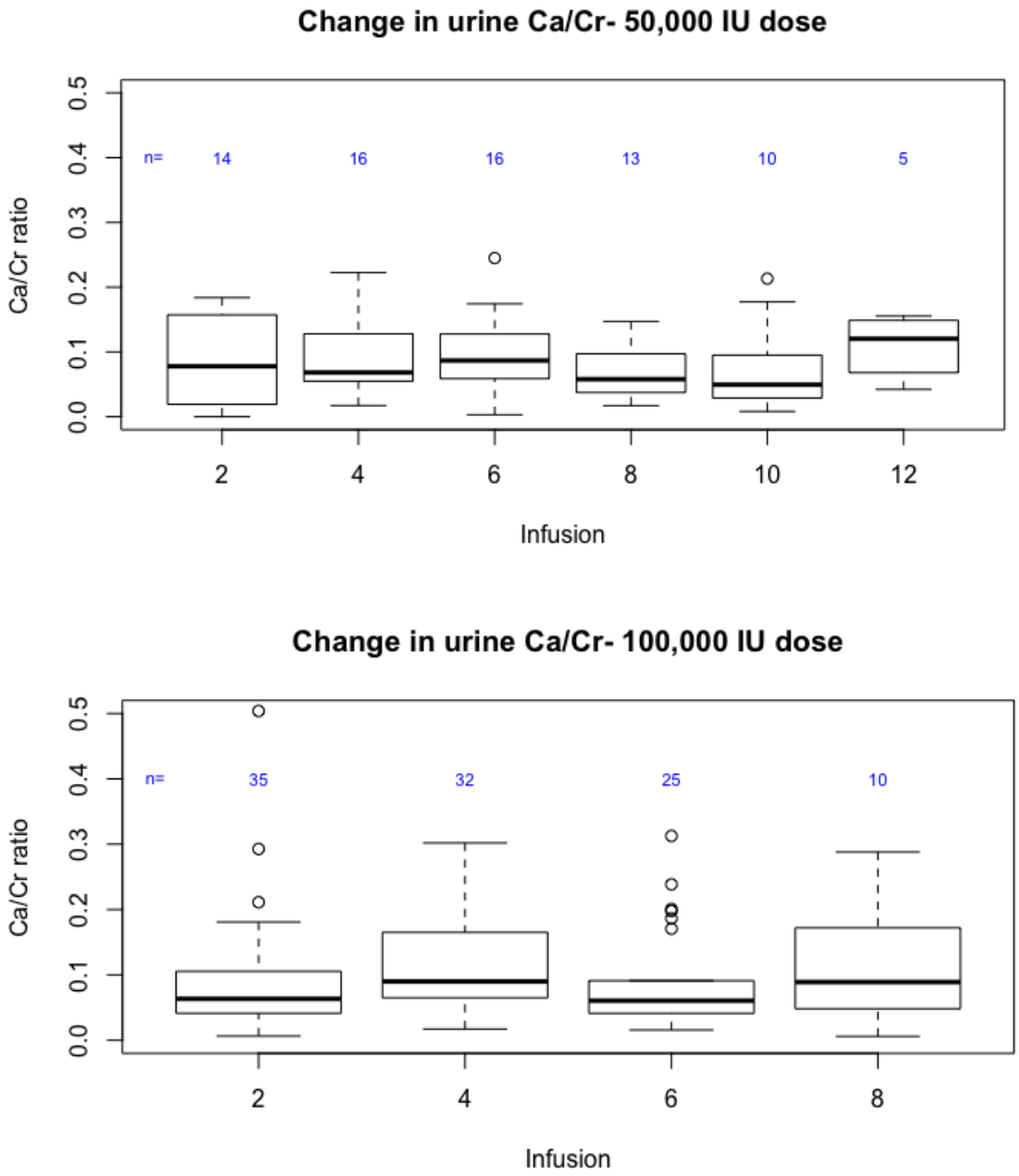

Figure 5. Effect of high dose therapy on urine calcium to creatinine ratios. Spot urine calcium to creatinine ratios were measured for the duration of high dose therapy in to monitor patient risk for hypercalciuria and renal stones. Mean Ca/Cr ratios were well below safety threshold (0.2) for both the 50,000 IU dosing group *(top)* and the 100,000 IU dosing group (*bottom)* throughout the study.

Spot urine Ca/Cr ratios were collected at every other Remicade infusion while patients were on the study to monitor patient risk of hypercalciuria as a result of vitamin D therapy. Follow-up urine samples were requested if a measured Ca/Cr was greater than 0.200 and a subsequent 24-hour urine collection was performed if both spot urine Ca/Cr ratios were greater than 0.200. Of the 60 patients who received three or more doses as part of the study, 10 (17%) had at least one measured urine Ca/Cr ratio > 0.200 and 5 (8%) had an elevated follow up Ca/Cr at a subsequent infusion. These 5 patients were then subject to 24-hour urine collections, all of which returned normal calcium to creatinine ratios (Table 6). There were no instances of kidney stones or signs of kidney dysfunction during the study period.

Table 6. Incidence of elevated urine calcium. Spot urine calcium/creatinine ratios taken over time were categorized as either "Normal" (≤0.200) or "Elevated" (>0.200). Subjects who recorded an elevated urine Ca/Cr had a follow up spot urine sample measured at their next infusion.

	Infusion 2	**Infusion 4**	**Infusion 6**	**Infusion 8**	**TOTAL**
50,000 IU	# OF SUBJECTS (%)				
Normal	14 (100%)	15 (94%)	15 (94%)	13 (100%)	57 (97%)
Elevated	0 (0%)	1 (6%)	1 (6%)	0 (0%)	2 (3%)
Elevated follow-up	--	0 (0%)	0 (0%)	--	0 (0%)
100,000 IU	# OF SUBJECTS (%)				
Normal	32 (91%)	27 (84%)	23 (88%)	9 (90%)	91 (88%)
Elevated	3 (9%)	5 (16%)	3 (12%)	1 (10%)	12 (12%)
Elevated follow-up	0 (0%)	2 (6%)	1 (4%)	0 (0%)	3 (3%)

No adverse events were observed during the study in regards to vitamin D therapy, nor did any enrolled subjects experience Remicade infusion reactions while on the study. It was noted that one consented patient had reported nausea with vitamin D

supplementation in the past and removed themselves from the study after experiencing similar symptoms upon their first dose. However, no such symptoms were reported by enrolled subjects for the remainder of the study.

Disease Activity

Baseline disease activity data was collected from 60 patients, including the subject's most recent aPCDAI or PUCAI, for patients with CD and UC, respectively. 44 patients with CD and all 14 UC patients had disease activity assessed within 8 weeks of their qualifying serum 25OHD level and before administration of the first study dose (disease activity metrics were not collected for patients with indeterminate colitis as aPCDAI or PUCAI were not applicable). 39 (88.6%) of subjects with aPCDAI data were classified as having inactive disease (aPCDAI < 10). Baseline serum 25OHD was 22.27 ± 4.77ng/mL in CD patients with inactive disease and 20.32 ± 4.38ng/mL in patients with mild or moderate-to-severe disease, respectively. An inverse correlation was observed between 25OHD levels and aPCDAI disease activity scores in this population with CD (R, -0.34; CI, -0.58 – -0.05; *p= .02*). Nine (64.3%) of subjects with UC had disease that was classified as being in remission (PUCAI< 10). Baseline serum 25OHD was 22.00 ± 5.64ng/mL in UC patients in clinical remission and 20.20 ± 6.81ng/mL in patients with mild, moderate, or severe disease. No significant correlation was found between 25OHD levels and PUCAI scores in subjects with UC (R, -0.28; CI, -0.71 – 0.29; P= 0.33).

As of February 1st, 2019, there were 12 subjects with CD, 4 subjects with UC, and 1 subject with Indeterminate colitis who had completed the one-year treatment protocol

and had available disease activity metrics available within 8 weeks of last vitamin D dose. Paired *t*-test analysis of disease activity scales, as reported by primary GI providers at follow up appointments, did not demonstrate any change in aPCDAI scores (*t*, 0.80; CI, -1.44 – 3.11; *p*= 0.44) for CD patients or any change in PUCAI scores (*t*, -1.57; CI, -11.37 – 3.87; *p*= 0.21) in UC patients. Observation and analysis of endpoint disease activity metrics will continue as more patients complete the study.

In addition to the starting point and endpoint disease activity metrics, inflammatory markers were recorded throughout the study protocol. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were collected at each Remicade infusion as part of regular clinical care. Serum markers of inflammation were not shown to be affected by vitamin D treatment up to steady-state 25OHD by paired *t*-test analysis for CRP (*t*, -0.17; CI, -0.04 – 0.04; *p*= 0.87) and ESR (*t*, 1.75; CI; -0.29 – 4.25; *p*= 0.09) between baseline levels and those at the fourth infusion. Changes in total 25OHD from baseline to steady state also did not appear to correlate with any changes in CRP (R, 0.16; CI, -0.13 – 0.42; *p*= 0.27) or ESR (R, -0.10; CI, -0.37 – 0.19; *p*= 0.51) over the first 3 doses (Figure 6).

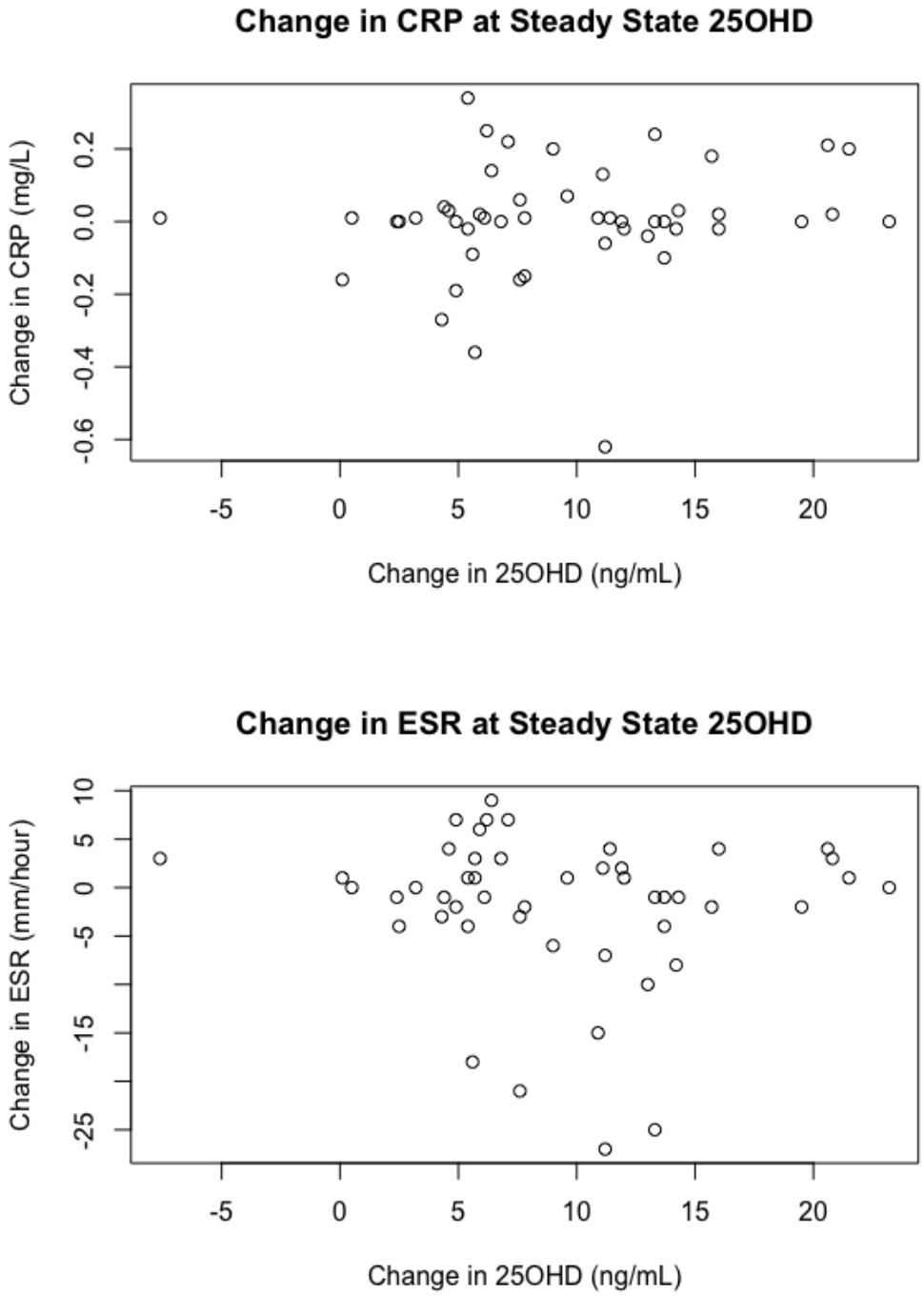

Figure 6. Effect of Vitamin D Status Change on Inflammatory Markers *In Vivo*. Serum inflammatory markers are tracked in IBD patients to monitor chronic inflammation. Changes in 25OHD levels from baseline to steady-state was not shown to associate with changes in CRP (*top)* or ESR (*bottom*).

Health Related Quality of Life (IMPACT-III)

A quality of life questionnaire (IMPACT-III) was completed by the subject and/or a guardian at the time of consent, which was within 8 weeks of a serum 25OHD < 30ng/mL. Two patients had HRQoL surveys that were not returned or provided within 8 weeks of qualifying lab date, leaving 59 patients with a reportable HRQoL metric. The average IMPACT-III score was 141.1. Self-reported quality of life did not appear to relate to vitamin D status, as no significant correlation was found between baseline 25OHD level and IMPACT-III score (R, 0.03; CI, -0.22 – 0.29; P= 0.79).

As of February 1st, 2019, there were 17 subjects who had completed the one-year treatment protocol and had provided both starting point and endpoint HRQoL data. Paired *t*-test analysis of IMPACT-III sub-scores, among both dosing groups, did not demonstrate any change in IBD related quality of life in patients receiving vitamin D treatment over the course of a year (Table 7).

Table 7. Change in HRQoL Metric Following One-Year Vitamin D Therapy. A standardized pediatric IBD HRQoL Survey was collected once at the time of consent and once again at last vitamin D dose, 48-64 weeks later. Paired *t*-test analysis does not suggest a change in IBD related quality of life among pediatric patients receiving interval vitamin D supplementation.

IMPACT-III [N=17]	***Mean Baseline***	***Mean Endpoint***	***Paired t-test value***	***95% Conf. interval***	***P-value***
Total	139.6±23.9	146.0±20.5	1.76	-1.35 to 13.62	0.10
Bowel Symptoms	27.9±5.3	28.7±4.6	1.14	-0.90 to 3.06	0.27
Systemic Symptoms	11.1±2.7	11.3±2.1	0.41	-0.98 to 1.45	0.69
Emotional Functioning	27.5±6.6	28.4±6.1	0.56	-2.1 to 3.61	0.58
Social Functioning	49.8±7.3	51.7±6.7	1.66	-0.48 to 3.85	0.12
Body Image	10.9±2.5	11.2±2.8	0.33	-1.02 to 1.39	0.74
Treatments and Interventions	12.5±2.2	13.3±1.4	2.06	-0.02 to 1.79	0.06

DISCUSSION

This study investigated the safety and efficacy of a novel intervention to manage vitamin D deficiency in pediatric patients with IBD. It is also the only study, to our knowledge, that provides a profile of confirmed oral vitamin D supplementation over multiple doses in a large sample of pediatric patients. In response to the disproportionate occurrence of vitamin D deficiency in IBD patients and the inability of prescribed supplementation to improve vitamin D status, this intervention used patients' regularly scheduled Remicade infusions as a framework to administer high dose vitamin D therapy. The dosing protocol was developed in the hopes of increasing vitamin D therapy adherence, in order to increase clinical efficacy, and making supplementation less cumbersome for patients and their families. For the purpose of investigation, this protocol conveniently allowed for meticulous observation of oral dose administration.

Study Sample Population

The investigation was conducted in a sample population of pediatric IBD patients with vitamin D deficiency. A sample population in Australia found 57% of children with IBD to have 25OHD < 30ng/mL, classified as either vitamin D deficient or insufficient (Levin et al., 2011). The current study screened over 500 IBD patients maintained on Remicade and found that 51% of patients to have 25OHD levels suggestive of vitamin D deficiency or insufficiency between Fall 2016 and Fall 2018. This highlights the prevalence of vitamin D deficiency in IBD patients living at temperate latitudes and emphasizes the importance of addressing vitamin D insufficiency in these patients.

Screening for our study also revealed that less than 10% of patients with vitamin D insufficiency or deficiency exhibited liver, kidney, or granulomatous disorders that might make high dose vitamin D supplementation inappropriate, and demonstrates that high dose vitamin D_3 supplementation is a viable vitamin D management option for the vast majority of pediatric IBD patients.

Efficacy

A sample of vitamin D deficient patients maintained on Remicade for IBD symptoms received interval doses of 50,000 IU or 100,000 IU, as determined by prescribed Remicade infusion frequency, of vitamin D_3 orally for approximately one year. Serial monitoring of serum 25OHD levels revealed that, on average, patients reached steady state vitamin D levels of 28.9 ng/mL and 33.2 ng/mL, for the 50,000 IU dosing group and 100,000 IU dosing groups respectively, after 3 doses, or 4-8 months after therapy initiation (Figure 3). Both dosing regimens achieved average 25OHD level increases of about 9 ng/mL that were found to be statistically significant. A pharmacokinetic study in adults that explored the effect of a single 100,000 IU dose of vitamin D_3 taken orally found that serum 25OHD levels peaked one week after dose administration, where 15 ng/mL increases from baseline were observed, and that 25OHD levels, on average remained elevated from baseline for about twelve weeks (Ilahi et al., 2008). The current study intended to see the effect of supplementation on steady-state vitamin D status, rather than peak and trough 25OHD levels, and therefore 25OHD levels were measured either 8 weeks, for the 50,000 IU dose group, or 16 weeks, for the

100,000 IU dose group, after the most recent dose administration. This shows that repeated interval supplementation is capable of more permanently re-establishing individuals' vitamin D level, resulting in increased 25OHD levels even after the response to a single dose would have run its course. The current investigation was the first study, to our knowledge, to monitor 25OHD level response to repeated administrations high dose vitamin D supplementation and therefore it is unknown why 25OHD levels appear to plateau and reach a steady-state after three doses. It is possible that, after three or four doses, the amount of vitamin D_3 simply overwhelmed the vitamin D 25-hydroxylase's ability to convert vitamin D_3 to 25OHD, limiting the rate of synthesis, and/or that feedback from rising $1,25(OH)_2D$ levels increased the degradation of 25OHD by 25OHD 24-hydroxylase, increasing rate the metabolism. Both mechanisms would result in a steady-state 25OHD level where the rate of 25OHD synthesis is equal to the total rate of 25OHD metabolism. However, for financial reasons, the current study did not monitor vitamin D_3 or $24,25(OH)_2D$ levels and was unable to detect any pooling of vitamin D_3 or changes in vitamin D metabolites that may have resulted from vitamin D_3 supplementation. Further investigation into vitamin D metabolism shifts *in vivo* in response to clinical supplementation are needed to illuminate the primary factor(s) influencing steady-state 25OHD levels. This evidence demonstrates the potential for high doses of vitamin D_3 administered at Remicade infusions to effectively improve pediatric patients' overall vitamin D status.

In agreement with the IRB, this intervention study was developed with the intention to treat and did not include a non-treatment or placebo control group. Therefore,

screening and recruitment was exclusive to patients who exhibited vitamin D insufficiency or deficiency. In this sample of vitamin D deficient or insufficient IBD patients living in New England, USA, there were about twice as many male subjects, three times as many subjects with CD than UC, and the subject population was 70% white. However, this demographic profile is not too dissimilar from that of the patients treated in BCH's IBD program, the population from which the sample was drawn, and therefore does not point to an increased risk of vitamin D deficiency in any particular demographic group. Analysis of baseline vitamin D status did reveal significantly lower 25OHD levels at baseline in subjects identifying their race as 'Other' when compared to subjects who chose one of the racial categories. It has been documented by other studies that darker skin complexions and identification as "black" are correlated with increased risk of vitamin D deficiency and decreased response to vitamin D supplementation, respectively (Pappa et al., 2006b; Lewis et al., 2013). However, self-identified race was not shown to influence subject response to therapy, as reported by change in 25OHD from baseline to steady-state, and subjects' reported sun sensitivity and tendency to tan was not found to correlate with lower baseline 25OHD or decreased response to therapy. While the potential for increased risk of vitamin D deficiency in populations with darker skin complexion should not be overlooked, the data presented here does not appear to suggest that race or skin complexion plays a significant role in the efficacy of vitamin D supplementation and is not expected to be a predictor of individual patient response to vitamin D therapy. Additionally, the severity of deficiency in baseline 25OHD levels was not shown to differ between genders, between CD and UC patients, or with age. None of

these factors were shown to affect steady state 25OHD levels or change in 25OHD from baseline to steady-state, and do not suggest that response to high dose therapy differs based upon gender, type of IBD, or age.

A meta-analysis of body composition and vitamin D status found that obese individuals, classified by BMI, were 35% more likely to be vitamin D deficient (Pereira-Santos et al., 2015). It is thought that obesity may result in low levels of circulating 25OHD because of the tendency for the fat-soluble vitamin D to sequester in the excess adipose tissue in obese patients. This study did not look at relative risk of obese patients suffering from vitamin D insufficiency or deficiency as no data was collected from vitamin D sufficient patients. In our population of interest, vitamin D deficient children and adolescents with IBD, there was no correlation with pretreatment 25OHD levels, steady-state 25OHD levels, or change in 25OHD from baseline and BMI. This suggests that BMI should not be a major factor of consideration when assessing the utility of high dose vitamin D supplementation in this clinical context.

Data on sun exposure and diet were also collected at time of consent and at each dose to monitor the effect behavioral changes on vitamin D status. 'Baseline' and 'Follow-Up' surveys collected information about how much time subjects spent outside on a typical day and how much of their skin was not covered by clothing (Appendices A & B). UVB exposure is considered a major factor influencing serum 25OHD levels: one study demonstrated that serum 25OHD levels increased 3-fold in subjects with lighter skin complexion and almost 2-fold in subjects with darker skin complexion after 12-weeks of UVB irradiation treatments (Chen et al., 2007) Baseline data did not suggest

that spending more time outside correlated with higher 25OHD levels, but rather revealed that increasing surface area of exposed skin, i.e. wearing a swimsuit rather than pants and a coat, was associated with higher pretreatment 25OHD levels (pages 17-18). The absorption of UVB radiation into the skin, which is often blocked by clothing, is required for the production of previtamin D3 by 7-dehydrocholesterol reductase in the epidermis. It would follow that the effect of time spent in the sun be diminished if most of the skin is covered by clothing, whereas increasing the amount of skin exposed to sunlight increases the rate of previtamin D3 synthesis for a set duration of time under the sun. However, neither of these factors were shown to affect the change in 25OHD in response to vitamin D_3 therapy and it is thought that the effect of high dose supplementation was significant enough to outweigh any effects on 25OHD that may be due to changes in endogenous vitamin D synthesis throughout the year. These effects are also considered seasonal as seasonal weather patterns dictate how people dress and how often they are outside.

Another reason for seasonal variation in epidermal previtamin D3 synthesis is the incident angle of sunlight hitting the earth. In locations further from the equator, the zenith angle of the sun decreases such that less solar radiation reaches the surface during the winter months. This means that in northern latitude locations in the United States, like Boston or Chicago, there is not enough UVB radiation reaching the earth's surface for any synthesis of vitamin D between November and February (Feldman, Krishnan, & Swami, 2013). Although seasonal variation in serum 25OHD levels has been demonstrated in populations living at these latitudes (Kroll et al., 2015b), there was no observed difference in pretreatment 25OHD level based on the month of treatment

initiation, which was within 8 weeks of baseline 25OHD measurement. Additionally, the time of year of the first three doses of the study was not shown to influence overall steady state 25OHD levels or the change in 25OHD from baseline (Figure 4). Due to the timing of the study's recruitment launch in October 2017 and a period of transition of coordinators, where recruitment efforts slowed, there were far more patients enrolled between the months of December and March. Perhaps this was also due in part to an increased number of patients falling below the study's deficiency threshold during the winter months. Nevertheless, supplementation appeared to improve vitamin D status throughout the year and demonstrated the potential to washout these seasonal effects.

The final source of vitamin D that was considered in order to evaluate changes in 25OHD was from subjects' diets. In the U.S. and Canada, milk and dairy products as well as many breakfast cereals are fortified with vitamin D; however, the most vitamin D rich sources that are readily available to people in North America are oily fish such as salmon, trout, cod, and haddock. Farmed salmon and trout contain three times as much vitamin D per ounce as cod or haddock, and about five to seven times as much as shellfish (Chen et al., 2007). Patients reported their typical consumption frequency of dairy, fortified cereals, and fish at their first dose and at each consecutive encounter. Relative consumption of vitamin D rich foods, based on patient report, was not shown to influence 25OHD levels at baseline or affect the response to supplementation from baseline to steady state for the 100,000 IU dosing group (Table 4). Effects of vitamin D rich diet were undeterminable for the 50,000 IU dosing group due to a small sample of completed dietary profiles. An unexpectedly large number of subjects were unsure as to whether or

not they consumed vitamin D fortified cereals and had to respond as such. Perhaps the vitamin and nutrient contents in fortified foods needs to be better presented on labeling and packaging, or consumers need to be more attentive to the nutritional contents of the foods they eat. While the responses to dietary habits appeared to range greatly between subjects, data on which food brands, sources, and quantity per serving were not collected such that dietary vitamin D could be estimated in IU/day for comparison to other studies of diet and vitamin D status. However, diets in the U.S. are generally considered to be low in vitamin D (Moore, Murphy, Keast, & Holick, 2004). There was no demonstrated effect of diet on 25OHD levels and no reason to suggest that dietary changes alone would be sufficient to improve vitamin D status in pediatric patients with IBD.

Safety

Serum chemistry labs were collected as part of normal clinical care in order to identify any unintended side-effects or increased risk associated with high dose therapy. For both dosing groups, there was no observed changes in serum calcium, creatinine, or BUN over the course of the study (Table 5). In the 100,000 IU dosing group, statistical analysis of serum phosphorus levels detected a decrease in subjects' serum phosphorus levels from pretreatment to their fourth vitamin D dose, about 6-8 months into therapy (Table 5). This same pattern was not observed in the 50,000 IU dosing group. Typically, the active form of vitamin D, $1,25(OH)_2D$, works to maintain serum phosphorus levels, by binding with VDR in the intestinal epithelium and in osteoblasts to increase the absorption of dietary phosphorus, mobilize phosphorus stores from mineralized bone, and

decrease wasting of phosphorus by the kidneys (Hossein-nezhad & Holick, 2013; Fukumoto, 2014). It seems counter-intuitive that increasing serum 25OHD, and presumably the amount of $1,25(OH)_2D$, would then decrease serum phosphorus. However, $1,25(OH)_2D$ synthesis and function is closely linked to that of parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23). PTH acts in synergy with $1,25(OH)_2D$ to mediate phosphorus and calcium transport out of bone and into circulation, but also opposes $1,25(OH)_2D$ action in the kidneys by blocking phosphate reabsorption and increasing urinary excretion of phosphate. While circulating PTH does contribute to decreases in phosphorus, FGF23 is a potent regulator of vitamin D that, in response to increased $1,25(OH)_2D$, inhibits 25OHD 1α-hydroxylase activity and tubular resorption of phosphate in the kidneys (Feldman et al., 2013). Therefore, it is probable that increases in $1,25(OH)_2D$, as a result of vitamin D_3 supplementation, induced relative increases in circulating FGF23 that acted on the kidneys to increase urinary excretion of phosphate and decrease serum phosphorus. However, neither PTH nor FGF23 nor $1,25(OH)_2D$ levels were collected as part of the study due to the expense and difficulty to acquire: circulating $1,25(OH)_2D$ is hard to measure because its concentration in plasma is .01% that of 25OHD (Feldman et al., 2013) and plasma FGF23 measurements have limited clinical utility due to high diurnal variability (Smith et al., 2012). While the current investigation detected a dip in serum phosphorus as a result of vitamin D therapy, there were no reported cases or manifestations of hypophosphatemia and, from a clinical standpoint, the administration of high doses of vitamin D_3 is not shown to cause significant disturbances or alterations in blood chemistry, most notably calcium and

phosphorus. More focused and higher-powered studies than the study conducted here are needed to extrapolate how high dose vitamin D supplementation may or may not affect phosphorus handling by PTH and FGF23 *in vivo*.

Although not commonly looked at in vitamin D supplementation studies, spot urine samples were also collected to regularly monitor hypercalciuria and assess patient risk for developing renal stones. Urine calcium-to-creatinine ratios were measured from spot urine samples at every other dose and found no overall change in urine calcium-to-creatinine ratios as a result of the first few high doses or after repeated intervals of high dose supplementation (Figure 5). 17% of subjects had at least one spot urine calcium-to-creatinine ratio found to be elevated over the course of the study, only 5% had elevated samples at consecutive encounters, and none of these returned elevated 24-hour urine calcium-to-creatinine ratios. Therefore, the elevated calcium-to-creatinine ratios measured are most likely a result of the subject having recently eaten a meal. It has been demonstrated that calcium to creatinine ratios calculated from spot urine collections do not correlate well with those calculated from 24-hour collection and are far less useful at identifying hypercalciuria. Such inconsistency in spot urine samples is due to the reactionary excretion of calcium by the kidneys in response to increased serum calcium directly following a meal, and results in overestimation of urine calcium-creatinine values post-prandially and underestimation during fasting (Jones et al., 2012). This investigation utilized spot urine collections given the convenience for subjects to provide samples at infusions, which are scheduled all throughout the day, and were merely used as a first-line of observation. The lack of elevated calcium-to-creatinine ratios from 24-hour

samples in this study demonstrates that vitamin D_3 administered in 50,000 and 100,000 IU doses does not increase patient risk for hypercalciuria or kidney stones in the short-term or over repeated doses.

Other than a single patient who opted to remove themselves from the study after they reported experiencing some nausea after their first dose there were no observed adverse events in relation to interval vitamin D therapy nor were there any Remicade infusion reactions in subjects while on the vitamin D study. Neuropsychiatric symptoms typically reported with vitamin D toxicity are confusion, drowsiness, and apathy secondary to hypercalcemia (Marcinowska-Suchowierska, et al., 2018) and it is not thought that the nausea symptoms reported were in response to the high dose of vitamin D_3, especially since the subject had reported experiencing these symptoms with other doses of vitamin D and because no other subjects appeared to present similar symptoms with the same dose. Perhaps this individual has a sensitivity to ingested vitamin D or has a sensitivity/allergy to one of the inactive ingredients in the vitamin D capsule, most commonly gelatin. The data from this investigation demonstrates that 50,000 IU and 100,000 IU doses of oral vitamin D given at four to eight-week intervals does not increase risk of hypercalcemia (Table 5) and hypercalciuria (Figure 5) in pediatric patients, and overall suggests this dosing regimen to be a minimal risk intervention.

Disease Activity and IBD Symptoms

One of the aims of this study was to assess the utility of a novel vitamin D therapy regimen as part of the clinical management of IBD in children and adolescents, therefore

disease activity scores, inflammatory biomarker levels, and an HRQoL survey were collected for each subject and related to 25OHD. Disease activity analysis was separated by IBD diagnosis because there exists one standardized disease activity quantification tool for CD, the aPCDAI, and one for UC, the PUCAI. For CD patients, baseline aPCDAI scores were found to inversely correlate with serum 25OHD, i.e. higher 25OHD levels were associated with lower disease activity and less severe symptoms. This suggests that vitamin D could play a protective role in CD, or perhaps that increased inflammation and more active disease results in physiological and/or behavioral changes that influence vitamin D status: malabsorption and decreased appetite due to inflammation in the GI tract could markedly reduce the influx of vitamin D from dietary sources, or perhaps the pain and fatigue associated with active disease lead to patients spending more time inside and out of the sun. These findings are consistent with previous work showing that higher 25OHD levels were inversely correlated with intestinal inflammation in pediatric CD and UC (Garg et al., 2013). The same relationship was not found in patients with UC in this study, as PUCAI scores were shown to be independent of 25OHD level at baseline. Disease activity scores were also assessed at the conclusion of the study, after about one year on high dose therapy. Statistical analysis of the endpoint data collected so far does show a significant change in disease activity scores following months of interval supplementation. However, only a handful of subjects have completed the protocol and the effect of supplementation on aPCDAI and PUCAI scores will not be determined until more subjects reach the one-year endpoint and the sample size grows larger.

There is adequate data from murine models and clinical studies in humans that circulating 25OHD plays a significant role in immune system regulation in children and adults, acting to promote the differentiation and function of regulatory T cells in order to subdue inflammation (Yin & Agrawal, 2014b; S. Wu et al., 2015; Looman Kirsten I.M. et al., 2017). Patients regularly have ESR and CRP measured as part of their standard IBD care at BCH in order to monitor overall inflammatory status. These values were followed in study subjects throughout the study, and there was no association found between changes in ESR or CRP and change in 25OHD from each of their baseline levels. Similar to another study focused on the relationship between vitamin D status and pediatric IBD, a correlation was not established between 25OHD levels and clinical markers of systemic inflammation (Garg et al., 2013). There continues to be a lack of evidence to support the ability of vitamin D to influence inflammatory disease processes in a clinical context. Translation from animal models to clinical efficacy is thought to remain difficult because of varying threshold values for each of the numerous targets of $1,25(OH)_2D$ in the body. In this case, it is thought that the average 9 ng/mL increase in 25OHD was not sufficient to significantly alter markers of inflammation or disease processes in CD and UC. Perhaps a 25OHD of greater magnitude would induce noticeable shifts in clinical disease presentation.

Analysis of baseline quality of life responses did not suggest any correlation between total IMPACT-III scores and 25OHD in a sample of vitamin D deficient patients; however, any correlation between IMPACT-III scores and baseline 25OHD levels for vitamin D sufficient patients was not investigated in this study. If threshold

values for 25OHD and its effect on gastrointestinal disease symptoms are in a range above 30 ng/mL, then any correlation between the two would not have been detectable with the sample population. As HRQoL responses are only collected twice, once at baseline and once more at the 48 to 64-week endpoint, less than one third of subjects have paired HRQoL scores. Similar to disease activity index data, paired *t* test analysis of the small sample size so far does not reveal a significant change in the IMPACT-III total score, or any of its six sub scores, as a result of 12 months of vitamin D therapy. A determinable effect of 25OHD levels on overall patient quality of life will not be found until more data has been collected, but currently it is offered that the magnitude of 25OHD increase was not enough to reveal detectable changes in the IMPACT-III metric.

Conclusions and Future Directions

Analysis of an almost complete dataset of subjects receiving high dose vitamin D supplementation concurrent with Remicade infusions has shown that oral administration of 50,000 IU and 100,000 IU doses of vitamin D_3 is a safe and effective method for improving 25OHD levels in pediatric IBD patients with vitamin D deficiency. Interval dosing was able to achieve steady state 25OHD levels ~30 ng/mL after three doses in both dosing groups and maintain 25OHD levels elevated from baseline for about 12 months. Supplementation was the only factor demonstrated to affect the change in 25OHD levels throughout the study, suggesting that high dose therapy can overcome seasonal dips in vitamin D and absence of vitamin D in the diet. Serum 25OHD levels increased an average of 9 ng/mL from baseline to steady-state, but this change in 25OHD

level does not appear to be sufficient to have marked effects on IBD symptoms, inflammatory markers, or overall quality of life in IBD patients. Higher target vitamin D levels may be needed to see clinical improvements relevant to IBD. It is proposed that increasing the supplementation dose or frequency should allow for attainment of 25OHD levels above 40 ng/mL, which have been shown to be protective against colon cancer (K. Wu et al., 2007; Jenab et al., 2010; McCullough et al., 2018), without substantially increasing risk of toxicity or other side-effects. Future investigations on the safety of larger doses of vitamin D_3 and achievement of higher target 25OHD levels are needed to confidently identify the role vitamin D therapy plays in the clinical maintenance of pediatric IBD.

It has been shown that vitamin D status, both pre- and post-induction, has been predictive of IBD response to anti-TNFα therapies, such as Remicade. Two studies in adult cohorts have suggested that increased 25OHD levels are associated with increased likelihood and duration of sustained remission on TNFα blockers (Winter et al., 2017; Zator et al., 2014). While the duration of Remicade therapy response was not an observed outcome in this study, it was noted that there was no occurrence of adverse events of Remicade infusion reactions for subjects while on vitamin D therapy. Similar investigations should be run in pediatric populations to better understand the vitamin D's potential role as an adjunct to increase the safety and efficacy of anti-TNFα drugs.

Finally, this study used regularly scheduled infusions as a framework for supplementation and limited our treatment population to those IBD patients maintained on a common, but specific, intravenous therapy. As a result, the protocol excluded a large

population of IBD patients who are also vitamin D deficient. An amended dosing plan should be developed and tested to accommodate IBD patients who do not receive Remicade as a maintenance therapy.

APPENDIX A

Baseline Survey Case Report Form

Clinical Trial of High Dose Interval Vitamin D Supplementation in Patients with IBD

Form 1 — Baseline Data — Version 03/02/17

SECTION A: GENERAL INFORMATION

A1. Study ID Number: ___ ___

A2. Screening Number ___ ___

A3. Date of Visit: ___ ___ / ___ ___ / ___ ___ ___ ___
M M D D Y Y Y Y

A4. Date of Birth: ___ ___ / ___ ___ / ___ ___ ___ ___
M M D D Y Y Y Y

A5. Initials of person completing form: ___ ___ ___

SECTION B: MEDICAL HISTORY

B1. Do you have any health problems other than IBD that you are regularly treated for?

Yes.....1

No.....0 *(Go to question B2).*

B1a. What health problem(s)?

Asthma.....1

Seizures.....2

Diabetes.....3

Arthritis.....4

Other.....99

B1ai. Specify: _____

B2. Are you currently taking any medications, including prescription medications, OTCs, vitamins, herbals?

Yes.....1

No.....0 *(Go to question B3)*

Study ID: ___ ___ — Version 03.17.09 — Page 1 of 7

B2a. What medication(s)?

		Yes	No
i.	Inhaled or oral steroids (Prednisone, Flovent, Advair)	1	0
ii.	Seizure medication (Depakote, Tegretol)	1	0
iii.	Vitamins (multivitamin, Viactive, Caltrate D)	1	0
iv.	Herbals	1	0
v.	Other	1	0
vi.	Specify		

B3. Have you been hospitalized in the past year?

Yes 1

No.....0 *(Go to question B4).*

B3a. Why were you hospitalized? _____

B4. Have you had surgery in the past year?

Yes.....1

No.....0 *(Go to question C1).*

B4a. Why did you have surgery? _____

SECTION C: RACE & ETHNICITY

C1. Would you say you are of Hispanic ethnicity?

Yes 1

No.....0

C2. Which of the following group(s) do you identify with the most?

White.....1

Black or African American.....2

Asian.....3

Native Hawaiian or Pacific Islander.....4

American Indian or Alaskan Native.....5

Other.....99

C2i. Specify: _____

SECTION D: SKIN SENSITIVITY & PIGMENTATION

D1. With regard to sensitivity to the sun, would you say you are…

Very sensitive to sun and always burn easily……1

Very sensitive to sun and always burn……2

Sensitive to sun and burn moderately……3

Moderately sensitive to sun and burn minimally……4

Minimally sensitive to sun and rarely burn……5

Insensitive to sun and never burn……6

D2. With regard to your skin pigmentation and tanning, would you say you…

Tan little or not at all, even with repeated sun exposure……1

Tan minimally with repeated sun exposure……2

Tan gradually ……3

Tan easily ……4

Tan profusely ……5

Tan extremely……6

SECTION E: VITAMIN D SURVEY

E1. In the past 30 days, how often did you have milk to drink or on cereal? This would include chocolate and other flavored milks, as well as hot cocoa and lattes made with milk. Would you say…

Never……1

Rarely – less than 1x per week……2

Sometimes – once per week or more but less than once per day……3

Daily – once per day……4

Multiple times per day – 2 or more times per day……5

Don't know……- 8

E2. Do you eat vitamin D fortified cereal? For example – Total, enriched rice cereals, etc.

Never……1

Rarely – less than 1x per week……2

Sometimes – once per week……3

Often – once per day……4

Don't know……- 8

E3. Do you eat fish?

Yes……1

No……0 *(Go to question E4)*

E3a. Do you eat any of these fish?

		Yes	No	Don't Know
i.	Salmon, mackerel, sardines, herring	1	0	- 8
ii.	shellfish	1	0	- 8
iii.	Other	1	0	- 8
iv.	Specify:			

E3b. How often do you eat fish?

Less than 1 time per week……1

1 – 2 times per week……2

3 – 4 times per week……3

5 – 7 times per week……4

E4. On a typical day, how much time do you spend outside? Would you say….

Half an hour or less per day……1

Between half an hour and 1 hour per day……2

Between 1 and 2 hours per day……3 *(Go to question E4b).*

More than 2 hours per day……4 *(Go to question E4b).*

E4a. Of the following, what is main the reason for spending little time outside?

Not enough time (too busy)……1
Threat of crime or harm……2
No parks or facilities in the area……3
Lack of interest……4
Cold/bad weather……5
Other……99
E4ai. Specify: _____

E4b. Do you wear sunscreen?

Yes……1
No……0

E4c. When outside, how exposed is your skin to the sun?

Minimally (face and neck)……1
Moderately (arms and legs)……2
Highly (bathing suit exposure) ……3

E6. Do you ever go to a tanning salon and lie in a tanning bed?

Yes……1
No……0 *(Go to question E8).*
E6a. How often?
Once per month or less……1
2-3 times per month……2
Once per week……3
Multiple times per week……4
E6b. How long do you tan per session?
Less than 5 minutes……1
6 - 10 minutes……2
11 - 15 minutes……3
16 minutes or more ……4

E8. In the past 12 weeks, have you traveled outside of New England?

Yes……1

No……0 *(Go to question E9)*

E8a. Did you travel somewhere warm?

Yes……1

No ……0

E8b. When outside, how exposed was your skin to the sun?

Minimally (face and neck)……1

Moderately (arms and legs)……2

Highly (bathing suit exposure) ……3

E8c. How many days did you spend time in the sun?

Less than 1 day……1

1-3 days……2

4-6 days……3

7-14 days……4

>14 days……5

E8d. How many hours per day on average did you spend time in the sun?

<1 hour……1

1-3 hours……2

> 3 hours……3

E8e. Did you wear sunscreen?

Yes……1

No……0

E9. In the last 2 months, did you ever take any vitamins before this study regularly (daily, weekly, bi-weekly)?

Yes……..1

No……0

E9a. What kind of vitamins?

		Yes	No
i.	Multivitamin	1	0
ii.	Vitamin D supplements	1	0
iii.	Calcium supplements (including Tums)	1	0
iv.	Calcium & vitamin D supplements (Viactiv, Caltrate D)	1	0
v.	Other	1	0
vi.	Specify:		

APPENDIX B

Follow-Up Survey Case Report Form

Clinical Trial of High Dose Interval Vitamin D Supplementation in Patients with IBD

Form 3 | Follow-Up Visit | Version 03.17.09

SECTION A: GENERAL INFORMATION

A1. Study ID Number: ___ ___

A2. Date of Visit: ___ ___ / ___ ___ / ___ ___ ___ ___
M M D D Y Y Y Y

A3. Date of Birth: ___ ___ / ___ ___ / ___ ___ ___ ___
M M D D Y Y Y Y

A4. Initials of person completing form: ___ ___ ___

SECTION B: VITAMIN D SURVEY

I would like to ask you a few questions about your nutrition and daily activities.

B1. In the past 30 days, how often did you have milk to drink or on cereal? This would include chocolate and other flavored milks, as well as hot cocoa and lattes made with milk. Would you say…

Never……1

Rarely – less than 1x per week……2

Sometimes – once per week or more but less than once per day……3

Daily – once per day……4

Multiple times per day – 2 or more times per day……5

Don't know……- 8

B2. Do you eat vitamin D fortified cereal? For example – Total, enriched rice cereals, etc.

Never……1

Rarely – less than 1x per week……2

Sometimes – once per week……3

Often – once per day……4

Don't know……- 8

Study ID: ___ ___ | Version 03.17.09 | Page 1 of 5

B3. Do you eat fish?

Yes……1

No……0 *(Go to question B4).*

B3a. Do you eat any of these fish?

		Yes	No	Don't Know
i.	Salmon, mackerel, sardines, herring	1	0	- 8
ii.	shellfish	1	0	- 8
iii.	Other	1	0	- 8
iv.	Specify:			

B3b. How often do you eat fish?

Less than 1 time per week……1

1 – 2 times per week……2

3 – 4 times per week……3

5 – 7 times per week……4

B4. On a typical day, how much time do you spend outside? Would you say….

Half an hour or less per day……1

Between half an hour and 1 hour per day……2

Between 1 and 2 hours per day……3 *(Go to question B4b).*

More than 2 hours per day……4 *(Go to question B4b).*

B4a. Of the following, what is main the reason for spending little time outside?

Not enough time (too busy)……1

Threat of crime or harm……2

No parks or facilities in the area……3

Lack of interest……4

Cold/bad weather……5

Other……99

B4ai. Specify: _____

B4b. Do you wear sunscreen?

Yes……1

No……0

B4c. When outside, how exposed is your skin to the sun?

Minimally (face and neck)……1

Moderately (arms and legs)……2

Highly (bathing suit exposure) ……3

B5. Do you ever go to a tanning salon and lie in a tanning bed?

Yes……1

No……0 *(Go to question B6).*

B5a. How often?

Once per month or less……1

2-3 times per month……2

Once per week……3

Multiple times per week……4

B5b. How long do you tan per session?

Less than 5 minutes……1

6 - 10 minutes……2

11 - 15 minutes……3

16 minutes or more ……4

B8. In the past 12 weeks, have you traveled outside of New England?

Yes……1

No……0 *(Go to question B11).*

B8a. Did you travel somewhere warm?

Yes……1

No ……0

B8b. When outside, how exposed was your skin to the sun?

Minimally (face and neck)……..1

Moderately (arms and legs)……..2

Highly (bathing suit exposure) ……3

B8c. How many days did you spend time in the sun?

Less than 1 day……...1

1-3 days……..2

4-6 days……...3

7-14 days……4

>14 days……..5

B8d. How many hours per day on average did you spend time in the sun?

<1 hour……1

1-3 hours……..…2

> 3 hours……3

B8e. Did you wear sunscreen?

Yes……...1

No……..0

B11. During the study, did you ever take any vitamins other than the study vitamin we provided you?

Yes……...1

No……0

B11a. What kind of vitamins?

		Yes	No
i.	Multivitamin	1	0
ii.	Vitamin D supplements	1	0
iii.	Calcium supplements (including Tums)	1	0
iv.	Calcium & vitamin D supplements (Viactiv, Caltrate D)	1	0
v.	Other	1	0
vi.	Specify:		

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